

Supplemental information

The chaperone domain BRICHOS prevents amyloid β -peptide CNS toxicity in *Drosophila melanogaster*

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Table S1: Lack of effects on longevity and locomotor activity of A β 42 or BRICHOS transgenes. Survey of p values for between-groups comparisons of longevity (in italics in the upper right side) and locomotor activity (underlined in the lower left side).

	W1118	UAS-A β 42x1	UAS-A β 42x2	UAS-BRICHOS
W1118		<i>0.6032</i>	<i>0.7709</i>	<i>0.2461</i>
UAS-A β 42x1	<u>0.7527</u>		<i>0.3286</i>	<i>0.0683</i>
UAS-A β 42x2	<u>0.3071</u>	<u>0.1649</u>		<i>0.3913</i>
UAS-BRICHOS	<u>0.6271</u>	<u>0.4092</u>	<u>0.5552</u>	

Log-rank test was used to calculate p values between groups for longevity measurements, and Mann Whitney test was used to calculate the p values between groups for locomotor activity measurements at day 25.

Table S2: Effects on longevity and locomotor activity of A β 42 and/or BRICHOS expression. Survey of p values for between-groups comparisons of longevity (in italics in the upper right side) and locomotor activity (underlined in the lower left side).

	Control (Gal4- <i>elav</i> ^{c155})	BRICHOS	A β 42x1	A β 42x2	A β 42x1 + BRICHOS	A β 42x2 BRICHOS
Control (Gal4- <i>elav</i> ^{c155})		<i>0.1831</i>	<i>< 0.0001</i>	<i>< 0.0001</i>	<i>0.1327</i>	<i>0.0019</i>
BRICHOS	<u>0.375</u>		<i>< 0.0001</i>	<i>< 0.0001</i>	<i>0.8403</i>	<i>0.0602</i>
A β 42x1	<u>< 0.0001</u>	<u>< 0.0001</u>		<i>< 0.0001</i>	<i>< 0.0001</i>	<i>0.0133</i>
A β 42x2	<u>< 0.0001</u>	<u>< 0.0001</u>	<u>< 0.0001</u>		<i>< 0.0001</i>	<i>< 0.0001</i>
A β 42x1 + BRICHOS	<u>0.0188</u>	<u>0.0002</u>	<u>0.0143</u>	<u>< 0.0001</u>		<i>0.0615</i>
A β 42x2 + BRICHOS	<u>0.0027</u>	<u>< 0.0001</u>	<u>0.0074</u>	<u>< 0.0001</u>	<u>0.8703</u>	

Log-rank test was used to calculate p values between groups for longevity measurements, and Mann Whitney test was used to calculate the p values between groups for locomotor activity measurements at day 25.

Supplementary figure legends

Supplementary figure 1. **Gene constructs inserted into the transgenic *Drosophila melanogaster* flies.** (A) The coding sequence for human A β 42, corresponding to position 672-713 in human APP, downstream of the signal peptide of *Drosophila* necrotic gene was inserted in the 2nd and/or the 3rd chromosome. The sequence coding for the linker and BRICHOS domain of proSP-C downstream of the signal peptide of human proSP-B gene was inserted at the 3rd chromosome. (B) Crossings were made so that the flies containing the genotypes A β 42x1, A β 42x2, BRICHOS, A β 42x1 + BRICHOS, and A β 42x2 + BRICHOS were obtained, which were then crossed with the Elav^{C155}-Gal4 driver fly line for pan-neuronal expression.

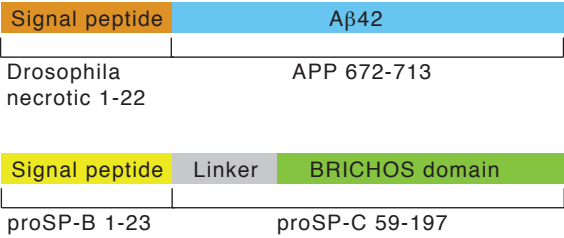
Supplementary figure 2. **BRICHOS expression does not affect A β 42 gene expression levels.** Elav^{C155}-Gal4 induced A β 42 mRNA expression levels in BRICHOS co-expressing flies was compared to levels in A β 42 only expressing flies using quantitative RT-PCR. (A) Relative mRNA levels in A β 42x1 and A β 42x1 + BRICHOS flies, and (B) relative mRNA levels of A β 42 in A β 42x2 and A β 42x2 + BRICHOS flies.

Supplementary figure 3. **Lack of effects on longevity and locomotor activity of A β 42 or BRICHOS transgenes.** (A) The fraction of 100 living flies over time is plotted for W1118 wildtype flies, UAS-A β 42x1, UAS-A β 42x2 and UAS-BRICHOS. Survival plots were calculated using the Kaplan-Meier method. (B) W1118 wildtype flies, UAS-A β 42x1, UAS-A β 42x2 and UAS-BRICHOS flies were analyzed in a climbing assay. Flies were analyzed at day 25 and the number of flies passing a line (8 cm above ground) within 10 sec were counted and expressed as percentage for each group of five flies. 50 flies of each genotype divided in 10 tubes were analyzed five times, and statistical analyses were made using the Mann Whitney test. The boxes extend from 25th to 75th percentiles and the whiskers are drawn from the 10th percentile to the 90th percentile.

Supplementary figure 4. **Negative controls for the confocal analyses.** (A) A dissected brain from a Gal4-*elav*^{C155} control fly was immunostained with anti-A β 42 antibody (green) and for

presynaptic zones (bruchpilot, red) and analyzed with confocal microscopy. The marked area in (A) is enlarged in images (B) to (E). (B) Only A β 42 staining, (C) staining for A β 42 and presynaptic zones. (D) Shows the staining using only the secondary antibody for anti-A β 42 (goat anti-rabbit Alexa Fluor 488) and (E) shows the same staining together with staining for presynaptic zones. Scale bar in (A) represents 200 nm.

A



B

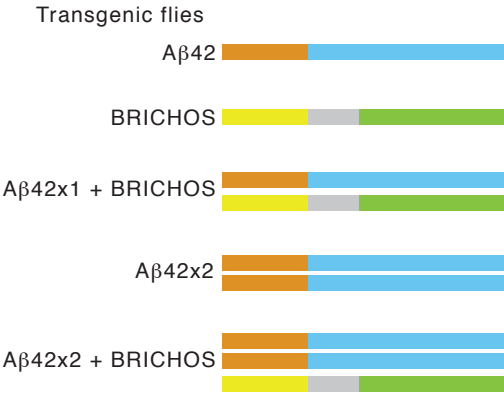


Fig. S1

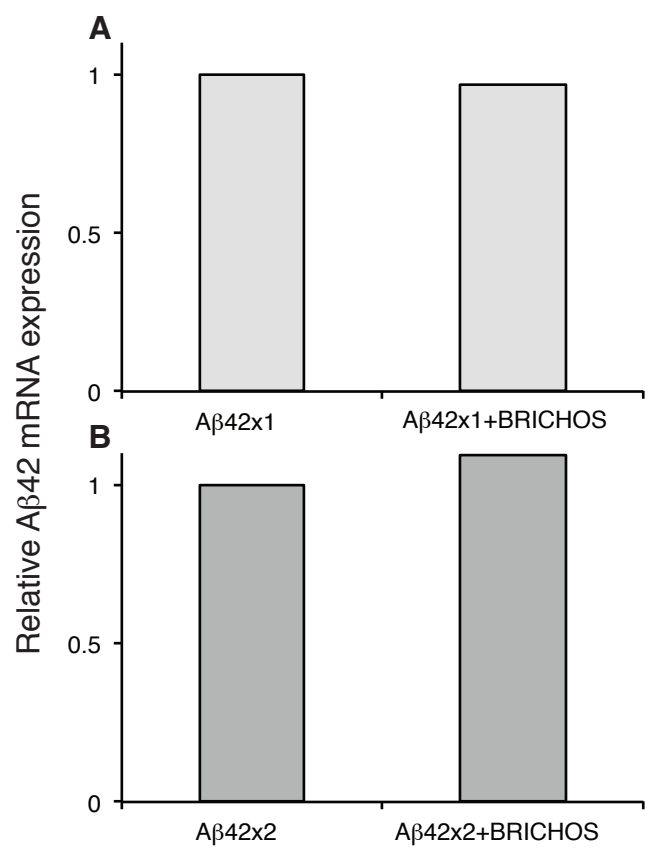


Fig. S2

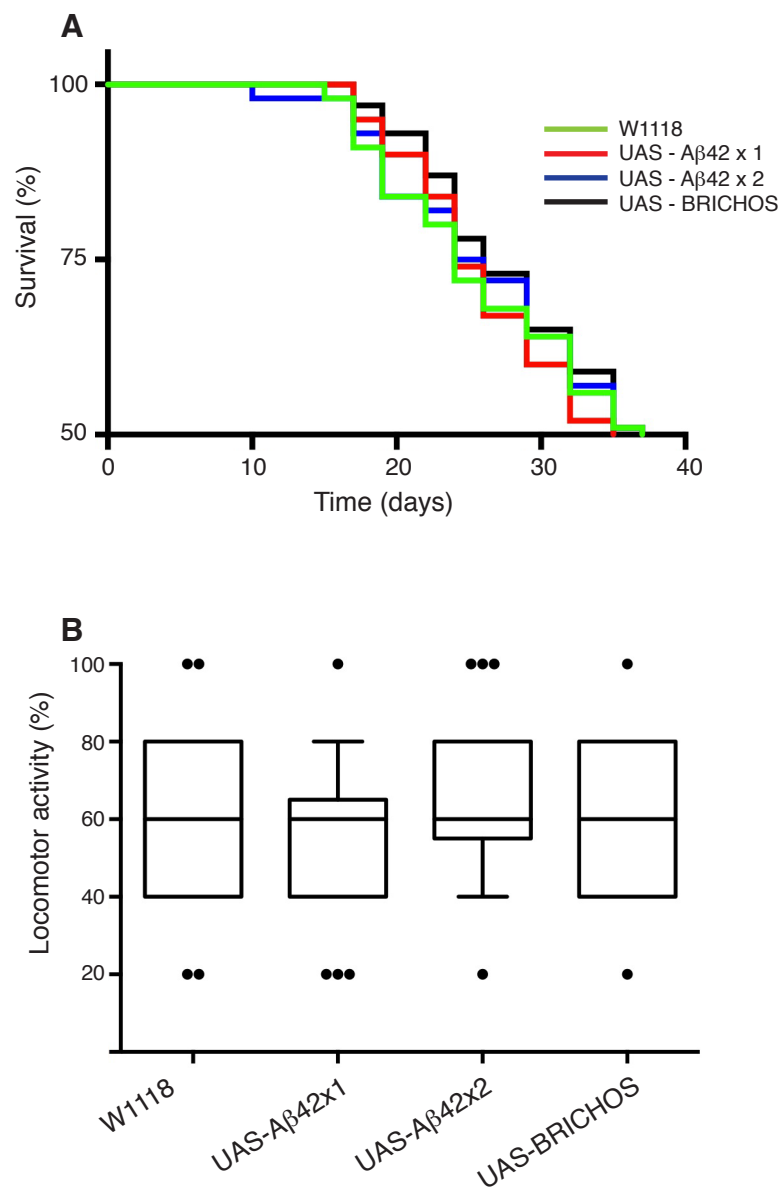


Fig. S3

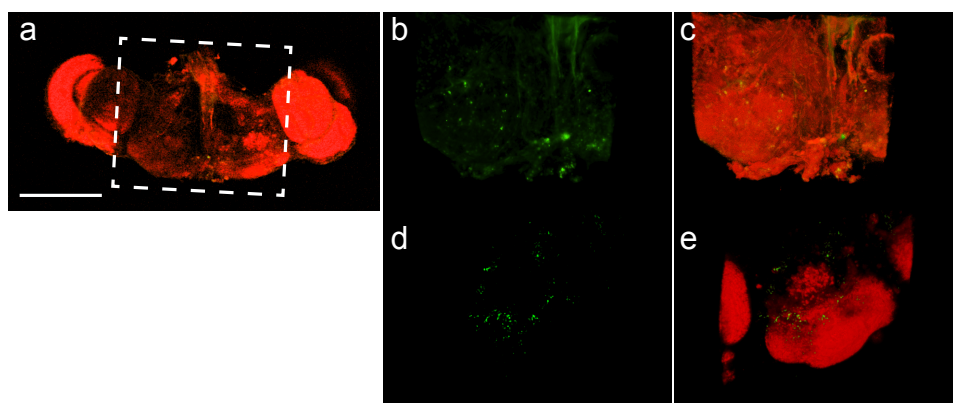


Fig. S4